## REMARKS/ARGUMENTS

Claims 49, 50, 59-72 and 77-90 are pending after the entry of this amendment. As a result of this amendment, claims 42-48, 53, 54, 58 and 73-76 are canceled without prejudice to renewal of the canceled subject matter in a related patent application.

Claims 49, 50 and 71 were amended to incorporate the limitations of canceled claim 58 or 73-76. Claims 59, 63, and 85 were amended to correct dependency or improve clarity. Claims 62 and 80 are amended to clarify that the multimer or multimerized fusion polypeptide is formed by interaction between the multimerizing components of two adjacent polypeptides. Support for this amendments is found in the specification at page 27, lines 23-25. Claims 89 and 90 are added to cover multimers that are tetrameric with respect to Ang-1-FD and Ang-2-FD, respectively. Support for these claims is found in the specification at page 24, line 31 to page 25, line 12. No new matter is added by this amendment.

### I. Objections to the Specification

Applicants were requested to replace the priority application at page 1, line 3. Accordingly, the specification is amended above to correctly state priority information.

### II. Claim Objections

Claims 47 and 48 were objected to various informalities. This objection is rendered moot by cancellation of the claims.

### III. Rejections Under 35 U.S.C. § 103(a)

Claims 42-52 and 55-88 were rejected as obvious over Pack et al. WO 96/37621 and further in view of Davis et al. U.S. Patent No. 6,265,564 and Desnick et al., U.S. Patent No. 5,580,757. Claims 42-48, 58, and 75-76 are cancelled. This rejection is respectfully traversed as applied and as is might be applied to the presently pending claims.

The Examiner has correctly recognized that none of the references by themselves teach the claimed invention. However, the rejection argues that the combination of the references would be obvious and that this combination at least taught the invention as claimed within the previously pending claims.

It is applicants' position that the combination of the references is not obvious particularly in that the primary reference to Pack et al. teaches away from the use of immunoglobulin-derived multimerizing domains shown at page 6, line 8 to page 8, line 5 of WO 96/37621. In view of the amendments made to the independent claims, applicants further argue that the use of the specific multimerizing components makes the combination of the references even more unobvious. However, notwithstanding applicants' position that the references do not provide a *prima facie* case of obviousness, it is applicants' position that the presently pending claims overcome any *prima facie* case of obviousness by demonstrating unexpected results. A more detailed discussion of the cases cited within the rejection, the art cited, specific distinguishing features between that art and the claimed invention such is put forth below.

The analysis under §103(a) requires that each cited prior art reference be assessed "as a whole" to determine (1) its scope and content, (2) the difference between the cited prior art reference and the claimed invention, and (3) the level of ordinary skill in the art at the time the invention was made. Graham v. John Deere Co., 383 U.S. 1, 148 USPQ 459 (1966). A rejection of a claim for obviousness over a combination of prior art references must establish that (1) the combination produces the claimed invention and (2) the prior art contains a suggestion or motivation to combine the references in such a way as to achieve the claimed invention. In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). A *prima facie* case of obviousness is potentially refutable with evidence of unexpected results or other objective indicia of nonobviousness. In re Soni, 54 F.3d 746, 34 USPQ2d 1684 (Fed. Cir. 1995).

The invention as claimed. Amended claim 49 is drawn to an isolated nucleic acid molecule encoding a fusion polypeptide comprising a first subunit comprising at least one copy of the receptor binding domain of angiopoietin-1, the first subunit being fused to the N-terminal end of a multimerizing component, and a multimerizing component being fused at its C-terminal end to a

second subunit comprising at least one copy of the receptor binding domain of angiopoietin-1, wherein the multimerizing component is an immunoglobulin derived domain selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG. Amended claim 50 is similar except drawn to a fusion polypeptide having the receptor binding domain of Ang-2 as a first and second subunit.

Claims 59-60 is drawn to a fusion polypeptide encoded by the isolated nucleic acid molecule of claim 49 or 50; claim 60 specifies that the fusion polypeptide is multimerized. Claim 61 is drawn to a composition comprising the multimerized fusion polypeptide of claim 60; claim 62 defines the multimer as a dimer. Claims 63-69 are vectors and host-vector systems, and claim 70 is drawn to a method of producing the fusion polypeptide.

Claim 71 is drawn to an isolated nucleic acid molecule encoding a fusion polypeptide comprising tandem receptor binding domains and a multimerizing component wherein the multimerizing component is an immunoglobulin derived domain selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG and the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2. Claim 77 is drawn to a fusion polypeptide encoded by the isolated nucleic acid molecule of claim 71; claim 78 specifies that the fusion polypeptide is multimerized. Claim 79 is drawn to a composition comprising the multimerized fusion polypeptide of claim 78; claim 80 defines the multimer as a dimer. Claims 81-87 are vectors and host-vector systems, and claim 88 is drawn to a method of producing the fusion polypeptide.

WO 96/37621 as a whole. Pack et al. describe multimeric polypeptides with two or more functional domains capable of self-multimerization. The invention is achieved with small peptidic multimerization devices, e.g., domain1-linker1-multimerization device-linker2-device2 (page 10, lines 13-14). Their peptidic multimerization device is 30-110 amino acids and capable of self-assembly. Functional domains may be from a general list, including domains that bind a defined target substance, catalyze a reaction, inhibit an enzyme, bind or block a receptor binding site, or bind a metal ion (page 22, lines 3-9). Experimental results with functional domains of antibody fragments show that tetramerization of bifunctional fusion proteins increased avidity (Example 1). Pack et al.

specifically teach away from use of immunoglobulin-derived multimerizing domains (see page 6, line 8 to page 8, line 5).

US 6,265,564 as a whole. Davis et al. teach fusion proteins that contain Ang-1-Fc and modified and chimeric Ang-1-Fc constructs capable of activating the TIE-2 receptor. It also describes an Ang-1 deletion variant that binds but does not activate the TIE-2 receptor (col. 10, lines 27-33); similarly an Ang-1 variant in which the fibrinogen-binding domain of Ang-1 is replaced with that of Ang-2 (col. 10, lines 40-45).

US 5,580,757 as a whole. Desnick et al. describes α-galactosidase – protein A fusion proteins.

The analysis under 35 USC § 103(a). The following analysis discusses the legal basis for finding that the Examiner has not established a *prima facie* case of obviousness:

1. Pack et al. is not combinable with Davis et al. because Pack et al. teach away from making the claimed invention.

Pack et al. teach against the use of immunoglobulin-derived multimerizing components because of glycosylations problems, expression problems caused by the enormous size of the CH2, CH3, CH4 domains, and folding efficiency caused by size. The invention of Pack et al. is presented as advantageous because of the features "of a relatively small size, low immunogenicity, and high yields of functional material" (page 8, lines 25-29) partially on the basis that the Pack et al. multimers do not contain an immunoglobulin –derived component.

The Federal Court of Appeals has specifically instructed that references cannot be combined when they "diverge from and teach away from the invention at hand" (In re Fine, 837 F.2d 1071, 5 USPQ2D 1596) (Fed. Cir. 1988); W. L. Gore & Assoc. v Garlock, Inc., 721 F.2d 1540, 1550, 220USPQ 303, 311) (Fed. Cir. 1983). Accordingly, Applicants submit that it is inconsistent with current patent case law to combine Pack et al.'s teaching against use of an Fc component with Davis et al.'s Ang1/2-Fc constructs.

2. Pack et al. do not teach forming a multimer with two or more functional domains from a protein that already exists in nature as two or more functional domains.

The problem addressed by Pack et al. is to enhance weak monovalent forces by multiplying the number of interactions (page 2, lines 15-17) to increase binding strength (page 2, lines 28-30). Multivalent interactions are taught as useful for achieving a synergistic gain in binding strength (page 2, lines 28-29) for functional domains that are found as single domains. Park et al. specifically distinguish their invention of the addition of a second functional domain from the teachings of the prior art (page 8, line 25 to page 9, line 4) ("A further limitation of WO 92/03569 is that it does not teach the preparation of multimers having two or more distinct functional domains per fusion protein") (page 8, lines 18-20).

Pack et al. is directed to enhancing binding of a functional domain to its target by increasing the number of interactions through multivalency. The functional domains described are generally a laundry list of "many different types of functional domains" which "may bind to a defined target substance, or catalyze reaction of a defined substance, or inhibit the action of an enzyme, or bind or block a receptor binding site, or bind to a metal ion" (page 22, lines 3-9) including antibody fragments and combinations of "unrelated functional domains such as enzymes, toxins, cytokines, kinases, phosphatases, lectins, peptide hormones, cell adhesion proteins including integrins, metal-binding domains, peptidic vaccines, bioactive peptides, or soluble cell surface proteins such as the CD molecules of leucocytes or parts thereof" (page 9, lines 10-21). The functional domains are characterized as domains that normally exist as a single component.

By contrast, angiopoietin-1 (ang-1) exists in nature as a modular structure consisting of a receptor binding domain, a dimerization motif, and a superclustering motif that forms variable-sized multimers. The fully assembled molecule is a hexamer consisting of two heterotrimeric building blocks ("the C domains of the fibrinogen α, β and γ chains form a coiled-coil structure, resulting in a heterotrimer. Two of these these heterotrimers are linked via interchain disulfide bonds among the cysteines contained within the N-terminal domains") (Davis et al. (2003) Nature Structural Biology 10:38-44) (copy enclosed).

Accordingly, Applicants submit that Pack et al. do not render the instant invention obvious because it does not teach the generation of a molecule having two or more functional domains for a molecule that already exists in nature with two or more functional domains.

3. Pack et al. do not teach a fusion protein having the same functional domains at each end.

Park et al. specifically distinguish their invention of the addition of a second functional domain from the teachings of the prior art (page 8, line 25 to page 9, line 4) ("A further limitation of WO 92/03569 is that it does not teach the preparation of multimers having two or more distinct functional domains per fusion protein") (page 8, lines 18-20).

4. Davis, et al. neither teaches nor suggests the activity of multimerized ligands in accordance with the present invention.

Davis, et al. describes the binding of Ang-1 fibrinogen domain-Fc dimers as well as anti-Fc antibody clustered preparations. However, one skilled in the art could not predict that such fibrinogen domains made as single chain dimers that self-multimerize would mimic the activity of the native Ang-1 and Ang-2 ligands; i.e. act as agonists or antagonists, respectively.

5. The engineered Ang1-Fc-Ang1 tetramer has a different functionality.

Angiopoietin-1 (Ang-1) acts via the endothelial receptor tyrosine kinase TIE2. As shown in Davis et al. (2003) *supra*, the ang-1 fibrinogen binding domain ($F_1$) (Fig. 1b, Davis et al. (2003) *supra*) does not activate TIE2 receptor in endothelial cells, nor does it activate endothelial TIE2 receptor as a dimer when fused to an Fc domain ($F_1$-Fc). However, the tetravalent molecule formed from multimerization of two $F_1$-Fc-$F_1$ molecules exhibits agonist activity. Accordingly, the engineered tetramer converts $F_1$ from an antagonist to an agonist.

While Pack et al. envisions enhancing the functionality of a molecule by increasing valency, nothing in Pack et al. teaches one of skill in the art to change an antagonist to an agonist.

6. The addition of Davis et al or Desnick et al. do not achieve the claimed invention.

Applicants respectfully submit that the combination of Pack et al. with either Davis et al. or Desnick et al. is irrelevant to the patentability of the instant claims as neither Davis et al. or Desnick et al. compensate for the Pack et al. instructions not to use an immunoglobulin-derived multimerizing component.

**Conclusion**

For the above reasons, Applicants submit that the Examiner has failed to establish a *prima facie* case of obviousness as required under the analysis required under § 103(a). In light of the above

amendments and arguments, Applicants contend that all claims are now in condition for allowance, and respectfully request such action.

**Fees**

The Final rejection was issued 17 March 2004. This amendment is filed with the accompanying RCE within the two month extension period ending 17 August 2004. Accordingly, a fee of $420 is due. Authorization is hereby given to charge the two month extension fee, and the amount of any additional fee to Deposit Account No. 18-0650.

Respectfully submitted

Valeta Gregg, Ph.D., Reg. No. 35,127
Regeneron Pharmaceuticals, Inc.
777 Old Saw Mill River Road
Tarrytown, New York 10591
(914) 345-7400

OIPE JC32 AUG 0 9 2004 PATENT & TRADEMARK OFFICE

## Includes Text of Claims being Canceled in this Amendment

Claims 1–41. Canceled

Claim 42. **(canceled)** An isolated nucleic acid molecule encoding a fusion polypeptide wherein the fusion polypeptide comprises a first subunit comprising at least one copy of a receptor binding domain of a ligand, the first subunit being fused to the N-terminal end of a multimerizing component, and the multimerizing component being fused at its C-terminal end to a second subunit comprising at least one copy of a receptor binding domain of a ligand, wherein the multimerizing component is an immunoglobulin-derived domain.

Claim 43. **(canceled)** The isolated nucleic acid molecule of claim 42, wherein the receptor binding domains of the first and second subunits are the receptor binding domain from the same ligand.

Claim 44. **(canceled)** The isolated nucleic acid molecule of claim 42, wherein the receptor binding domain of the first subunit is a receptor binding domain derived from a different ligand than the receptor binding domain of the second subunit.

Claim 45. **(canceled)** The isolated nucleic acid molecule of claim 43, wherein the receptor binding domain of the first and second subunit is the fibrinogen domain of angiopoietin-1.

Claim 46. **(canceled)** The isolated nucleic acid molecule of claim 43, wherein the receptor binding domain of the first and second subunit is the fibrinogen domain of angiopoietin-2.

Claim 47. **(canceled)** The isolated nucleic acid molecule of claim 44, wherein the receptor binding domain of the first subunit is the fibrinogen domain ~~is~~ of angiopoietin-1 and the receptor binding domain of the second subunit is the fibrinogen domain of angiopoietin-2.

Claim 48. **(canceled)** The isolated nucleic acid molecule of claim 44, wherein the receptor binding domain of the first subunit is the fibrinogen domain of angiopoietin-2 and the receptor binding domain of the second subunit is the fibrinogen domain of angiopoietin-1.

Claim 49. (currently amended) An isolated nucleic acid molecule encoding a fusion polypeptide wherein the fusion polypeptide comprises a first subunit ~~comprising~~ consisting of at least one copy of the receptor binding domain of angiopoietin-1, the first subunit being fused to the N-terminal end of a multimerizing component, and the multimerizing component being fused at its C-terminal end to a second subunit comprising at least one copy of the receptor binding domain of angiopoietin-1, wherein the multimerizing component is an immunoglobulin derived domain selected from the group consisting of the Fc domain of IgG and the heavy chain of IgG.

Claim 50. (currently amended) An isolated nucleic acid molecule encoding a fusion polypeptide wherein the fusion polypeptide comprises a first subunit ~~comprising~~ consisting of at least one copy of a receptor binding domain of angiopoietin-2, the first subunit being fused to the N-terminal end of a multimerizing component, and the multimerizing component being fused at its C-terminal end to a second subunit comprising at least one copy of the receptor binding domain of angiopoietin-2, wherein the multimerizing component is an immunoglobulin derived domain selected from the group consisting of the Fc domain of IgG and the heavy chain of IgG.

Claims 51-52. (Canceled)

Claim 53. (Withdrawn) The isolated nucleic acid molecule of claim 43, wherein the ligand is selected from the group consisting of the EPH family of ligands.

Claim 54. (Withdrawn) The isolated nucleic acid molecule of claim 44, wherein the ligands are selected from the group consisting of the EPH family of ligands.

Claims 55-57. (Canceled)

Claim 58. **(canceled)** The isolated nucleic acid molecule of claim 42, wherein the immunoglobulin derived domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.

Claim 59. (currently amended) A fusion polypeptide encoded by the isolated nucleic acid molecule of claim 49 or 50 ~~claims 42, 43, or 44~~.

Claim 60. (previously presented) The fusion polypeptide of claim 59, wherein the fusion polypeptide is multimerized.

Claim 61. (Previously presented) A composition comprising the multimerized fusion polypeptide of claim 60.

Claim 62. (currently amended) The composition of claim 61, wherein the multimer is a dimer formed by interaction between the multimerizing components of two adjacent fusion polypeptide molecules.

Claim 63. (currently amended) A vector which comprises the isolated nucleic acid molecule of claim 49 or 50 ~~claims 42, 43, or 44~~.

Claim 64. (currently amended) An expression vector comprising an isolated nucleic acid molecule of ~~claims 42, 43, or 44~~ claim 49 or 50, wherein the nucleic acid molecule is operatively linked to an expression control sequence.

Claim 65. (Previously presented) A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 64, in a suitable host cell.

Claim 66. (Previously presented) The host-vector system of claim 65, wherein the suitable host cell is a bacterial cell, yeast cell, insect cell or mammalian cell.

Claim 67. (Previously presented) The host-vector system of claim 66, wherein the suitable host cell is *E. coli.*

Claim 68. (Previously presented) The host-vector system of claim 66, wherein the suitable host cell is a COS cell.

Claim 69. (Previously presented) The host-vector system of claim 66, wherein the suitable host cell is a CHO cell.

Claim 70. (Previously presented) A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claim 66, under conditions permitting production of the fusion polypeptide and recovering the polypeptide so produced.

Claim 71. (currently amended) An isolated nucleic acid molecule encoding a fusion polypeptide, wherein the fusion polypeptide ~~comprises~~ consisting of more than one copy of a receptor binding domain of a ligand, each copy fused in tandem, and wherein either the N-terminal or the C-terminal ends of the tandem receptor binding domains is fused to a multimerizing component, wherein the multimerizing component is an immunoglobulin-derived domain selected from the group consisting of the Fc domain of IgG and the heavy chain of IgG.

Claim 72. (previously amended) The isolated nucleic acid molecule of claim 71, wherein the receptor binding domains are fused contiguously.

Claim 73. (previously amended) The isolated nucleic acid molecule of claim 71 or 72, wherein the ligand is not a member of the EPH family of ligands.

Claim 74. (previously amended) The isolated nucleic acid molecule of claim 71 or 72, wherein the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2.

Claim 75. **(canceled)** The isolated nucleic acid molecule of claim 71 or 72, wherein the multimerizing component comprises an immunoglobulin derived domain.

Claim 76. **(canceled)** The isolated nucleic acid molecule of claim 71, wherein the immunoglobulin derived domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.

Claim 77. (previously presented) A fusion polypeptide encoded by the isolated nucleic acid molecule of claims 71.

Claim 78. (previously presented) The fusion polypeptide of claim 77, wherein the fusion polypeptide is multimerized.

Claim 79. (previously presented) A composition comprising the multimerized fusion polypeptide of claim 78.

Claim 80. (currently amended) The composition of claim 79, wherein the multimerized fusion polypeptide is a dimer formed by interaction between the multimerizing components of two adjacent fusion polypeptide molecules.

Claim 81. (previously presented) A vector which comprises the isolated nucleic acid molecule of claim 71.

Claim 82. (previously presented) An expression vector comprising a nucleic acid molecule of claim 71, wherein the nucleic acid molecule is operatively linked to an expression control sequence.

Claim 83. (previously presented) A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 82, in a suitable host cell.

Claim 84. (previously presented) The host-vector system of claim 83, wherein the suitable host cell is a bacterial cell, yeast cell, insect cell or mammalian cell.

Claim 85. (currently amended) The host-vector system of claim 84, wherein the suitable host cell is ~~E. coli~~ *E. coli*.

Claim 86. (previously presented) The host-vector system of claim 84, wherein the suitable host cell is a COS cell.

Claim 87. (previously presented) The host-vector system of claim 84, wherein the suitable host cell is a CHO cell.

Claim 88. (previously presented) A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claim 83, under conditions permitting production of the fusion polypeptide and recovering the polypeptide so produced.

Claim 89. (new) The composition of claim 61 wherein the multimer is tetrameric with respect to Ang1-FD.

Claim 90. (new) The composition of claim 61 wherein the multimer is tetrameric with respect to Ang2-FD.